

Amendments to the Specification:

Please amend the specification by replacing the paragraph on page 10, lines 9-14, with the following amended paragraph:

Figures 5 A and 5 B are representations of firefly luciferase and cyclophilin siRNA panels sorted according to functionality and predicted values using Formula VIII. The siRNA found within the circle represent those that have Formula VIII values (SMARTscoreTM SMARTSCORETM, or siRNA rank) above zero. SiRNA outside the indicated area have calculated Formula VIII values that are below zero. Y-axis is "Expression (% Control)." Each position on the X-axis represents a unique siRNA.

Please amend the specification by replacing the paragraph on page 11, lines 4-6, with the following amended paragraph:

Figure 9 shows a graph of SMARTscoreTM SMARTSCORETM, or siRNA rank, versus RNAi silencing values for more than 360 siRNA directed against 30 different genes. SiRNA to the right of the vertical bar represent those siRNA that have desirable SMARTscoreTM SMARTSCORETM, or siRNA rank.

Please amend the specification by replacing the paragraph on page 16, lines 16-20, with the following amended paragraph:

SMARTscoreTM SMARTSCORETM, or siRNA rank

The term "SMARTscoreTM SMARTSCORETM, or siRNA rank" refers to a number determined by applying any of the Formulas I - Formula IX to a given siRNA sequence. The term "SMART-selected" or "rationally selected" or "rational selection" refers to siRNA that have been selected on the basis of their SMARTscoreTM SMARTSCORETM, or siRNA ranking.

Please amend the specification by replacing the paragraph on page 26, lines 4-29, with the following amended paragraph:

Additionally, in many applications, more than one of these formulas would provide useful information as to the relative functionality of potential siRNA sequences. However, it is beneficial to have more than one type of formula, because not every formula will be able to help to differentiate among potential siRNA sequences. For example, in particularly high GC mRNAs, formulas that take that parameter into account would not be useful and application of formulas that lack GC elements (*e.g.*, formulas V and VI) might provide greater insights into duplex functionality. Similarly, formula II might be used in situations where hairpin structures are not observed in duplexes, and formula IV might be applicable for sequences that have higher AU content. Thus, one may consider a particular sequence in light of more than one or even all of these algorithms to obtain the best differentiation among sequences. In some instances, application of a given algorithm may identify an unusually large number of potential siRNA sequences, and in those cases, it may be appropriate to re-analyze that sequence with a second algorithm that is, for instance, more stringent. Alternatively, it is conceivable that analysis of a sequence with a given formula yields no acceptable siRNA sequences (*i.e.* low SMARTscores™ SMARTSCORES™, or siRNA ranking). In this instance, it may be appropriate to re-analyze that sequences with a second algorithm that is, for instance, less stringent. In still other instances, analysis of a single sequence with two separate formulas may give rise to conflicting results (*i.e.* one formula generates a set of siRNA with high SMARTscores™ SMARTSCORES™, or siRNA ranking, while the other formula identifies a set of siRNA with low SMARTscores™ SMARTSCORES™, or siRNA ranking). In these instances, it may be necessary to determine which weighted factor(s) (*e.g.* GC content) are contributing to the discrepancy and assessing the sequence to decide whether these factors should or should not be included. Alternatively, the sequence could be analyzed by a third, fourth, or fifth algorithm to identify a set of rationally designed siRNA.

Please amend the specification by replacing the paragraph on page 36, line 27 to page 37, line 6, with the following amended paragraph:

SiRNA identified and optimized in this document work equally well in a wide range of cell types. **Figure 3a** shows the evaluation of thirty siRNAs targeting the DBI gene in three cell lines derived from different tissues. Each DBI siRNA displays very similar functionality in HEK293 (ATCC, CRL-1573, human embryonic kidney), HeLa (ATCC, CCL-2, cervical epithelial adenocarcinoma) and DU145 (HTB-81, prostate) cells as determined by the B-DNA assay. Thus, siRNA functionality is determined by the primary sequence of the siRNA and not by the intracellular environment. Additionally, it should be noted that although the present invention provides for a determination of the functionality of siRNA for a given target, the same siRNA may silence more than one gene. For example, the complementary sequence of the silencing siRNA may be present in more than one gene. Accordingly, in these circumstances, it may be desirable not to use the siRNA with highest ~~SMARTscore™~~ SMARTSCORE™, or siRNA ranking. In such circumstances, it may be desirable to use the siRNA with the next highest ~~SMARTscore™~~ SMARTSCORE™, or siRNA ranking.

Please amend the specification by replacing the paragraph on page 40, line 27 to page 41, line 2, with the following amended paragraph:

In an effort to improve selection further, all identified criteria, including but not limited to those listed in Table IV were combined into the algorithms embodied in Formula VIII and Formula IX. Each siRNA was then assigned a score (referred to as a ~~SMARTscore™~~ SMARTSCORE™, or siRNA ranking) according to the values derived from the formulas. Duplexes that scored higher than 0 or 20, for Formulas VIII and IX, respectively, effectively selected a set of functional siRNAs and excluded all non-functional siRNAs. Conversely, all duplexes scoring lower than 0 and 20 according to formulas VIII and IX, respectively, contained some functional siRNAs but included all

non-functional siRNAs. A graphical representation of this selection is shown in **Figure 5**.

Please amend the specification by replacing the paragraph on page 53, line 15 to page 54, line 10, with the following amended paragraph:

Identification of hyperfunctional siRNA involves multiple steps that are designed to examine an individual siRNA agent's concentration- and/or longevity-profiles. In one non-limiting example, a population of siRNA directed against a single gene are first analyzed using the previously described algorithm (Formula VIII). Individual siRNA are then introduced into a test cell line and assessed for the ability to degrade the target mRNA. It is important to note that when performing this step it is not necessary to test all of the siRNA. Instead, it is sufficient to test only those siRNA having the highest ~~SMARTscores™~~ SMARTSCORES™, or siRNA ranking (*i.e.* ~~SMARTscore™~~ SMARTSCORE™ > -10). Subsequently, the gene silencing data is plotted against the ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings (see **Figure 9**). SiRNA that (1) induce a high degree of gene silencing (*i.e.* they induce greater than 80% gene knockdown) and (2) have superior ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings (*i.e.* a ~~SMARTscore™~~ SMARTSCORE™ of > -10, suggesting a desirable average internal stability profile) are selected for further investigations designed to better understand the molecule's potency and longevity. In one, non-limiting study dedicated to understanding a molecule's potency, an siRNA is introduced into one (or more) cell types in increasingly diminishing concentrations (*e.g.* 3.0 → 0.3 nM). Subsequently, the level of gene silencing induced by each concentration is examined and siRNA that exhibit hyperfunctional potency (*i.e.* those that induce 80% silencing or greater at *e.g.* picomolar concentrations) are identified. In a second study, the longevity profiles of siRNA having high (>-10) ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings, and greater than 80% silencing are examined. In one non-limiting example of how this is achieved, siRNA are introduced into a test cell line and the levels of RNAi are measured over an extended period of time (*e.g.* 24-168 hrs). SiRNAs that exhibit strong RNA interference patterns

(*i.e.* >80 % interference) for periods of time greater than, *e.g.*, 120 hours are thus identified. Studies similar to those described above can be performed on any and all of the $>10^6$ siRNA included in this document to further define the most functional molecule for any given gene. Molecules possessing one or both properties (extended longevity and heightened potency) are labeled “hyperfunctional siRNA,” and earmarked as candidates for future therapeutic studies.

Please amend the specification by replacing the paragraph on page 54, line 18 to page 55, line 5, with the following amended paragraph:

The highest quality siRNA achievable for any given gene may vary considerably. Thus, for example, in the case of one gene (gene X), rigorous studies such as those described above may enable the identification of an siRNA that, at picomolar concentrations, induces 99+% silencing for a period of 10 days. Yet identical studies of a second gene (gene Y) may yield an siRNA that at high nanomolar concentrations (*e.g.* 100nM) induces only 75% silencing for a period of 2 days. Both molecules represent the very optimum siRNA for their respective gene targets and therefore are designated “hyperfunctional.” Yet due to a variety of factors including but not limited to target concentration, siRNA stability, cell type, off-target interference, and others, equivalent levels of potency and longevity are not achievable. Thus, for these reasons, the parameters described in the before mentioned assays, can vary. While the initial screen selected siRNA that had ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings, above -10 and a gene silencing capability of greater than 80%, selections that have stronger (or weaker) parameters can be implemented. Similarly, in the subsequent studies designed to identify molecules with high potency and longevity, the desired cutoff criteria (*i.e.* the lowest concentration that induces a desirable level of interference, or the longest period of time that interference can be observed) can vary. The experimentation subsequent to application of the rational criteria of this application is significantly reduced where one is trying to obtain a suitable hyperfunctional siRNA for, for example, therapeutic use. When, for example, the additional experimentation of the type described

herein is applied by one skilled in the art with this disclosure in hand, a hyperfunctional siRNA is readily identified.

Please amend the specification by replacing the paragraph on page 64, lines 3-23, with the following amended paragraph:

The algorithm (Formula VIII) identified siRNAs for five genes, human DBI, firefly luciferase (fLuc), renilla luciferase (rLuc), human PLK, and human secreted alkaline phosphatase (SEAP). Four individual siRNAs were selected on the basis of their SMARTscores™ SMARTSCORES™, or siRNA rankings, derived by analysis of their sequence using Formula VIII (all of the siRNAs would be selected with Formula IX as well) and analyzed for their ability to silence their targets' expression. In addition to the scoring, a BLAST search was conducted for each siRNA. To minimize the potential for off-target silencing effects, only those target sequences with more than three mismatches against un-related sequences were selected. Semizarov, *et al*, *Specificity of short interfering RNA determined through gene expression signatures*. Proc. Natl. Acad. Sci. U.S.A. 2003, 100:6347. These duplexes were analyzed individually and in pools of 4 and compared with several siRNAs that were randomly selected. The functionality was measured a percentage of targeted gene knockdown as compared to controls. All siRNAs were transfected as described by the methods above at 100 nM concentration into HEK293 using Lipofectamine 2000. The level of the targeted gene expression was evaluated by B-DNA as described above and normalized to the non-specific control. **Figure 10** shows that the siRNAs selected by the algorithm disclosed herein were significantly more potent than randomly selected siRNAs. The algorithm increased the chances of identifying an F50 siRNA from 48% to 91%, and an F80 siRNA from 13% to 57%. In addition, pools of SMART siRNA silence the selected target better than randomly selected pools (see Figure 10F).

Please amend the specification by replacing the paragraph on page 69, lines 8-14, with the following amended paragraph:

To identify functional and hyperfunctional siRNA against the Bcl2 gene, the sequence for Bcl-2 was downloaded from the NCBI Unigene database and analyzed using the Formula VIII algorithm. As a result of these procedures, both the sequence and ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings, of the Bcl2 siRNA were obtained and ranked according to their functionality. Subsequently, these sequences were BLAST'ed (database) to insure that the selected sequences were specific and contained minimal overlap with unrelated genes. The ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings, for the top 10 Bcl-2 siRNA are identified in **Figure 13**.

Please amend the specification by replacing the paragraph on page 69, lines 17-23, with the following amended paragraph:

Bcl-2 siRNAs having the top ten ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings, were selected and tested in a functional assay to determine silencing efficiency. To accomplish this, each of the ten duplexes were synthesized using 2'-O-ACE chemistry and transfected at 100nM concentrations into cells. Twenty-four hours later assays were performed on cell extracts to assess the degree of target silencing. Controls used in these experiments included mock transfected cells, and cells that were transfected with a non-specific siRNA duplex.

Please amend the specification by replacing the paragraph on page 74, lines 6-26, with the following amended paragraph:

With respect to the disks, there are four tables on each disk copy in text format: Tables XII –XV. Table XII, which is located in a file entitled Table_12.txt, provides a list of the 80-100 sequences for each target, identified by Formula VIII as having the

highest relative ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings, for the target analyzed. Table XIII, which is located in a file entitled Table_13.txt, provides the ~~SMARTscores™~~ SMARTSCORES™, or siRNA rankings, and for each gene, a pool pick of up to four sequences is denoted. (The denotation of “1” in Table XIII means that it is a pool pick.) These pool pick sequences represent the most functional siRNAs for the corresponding target. Any 1, 2, 3, or 4 of the pool pick sequences could be used for gene silencing. Further, sequences that are not denoted as pool pick sequences, but that are included on the compact disks may also be used for gene silencing either alone or in combination with other sequences. However, their individual relative functionality would be less than that of a pool pick sequence. Table XIV, which is located in a file entitled Table_14.txt, provides an identification of genes by accession number, and Table XV, which is located in a file entitled Table_15.txt, provides a short name for the genes identified on the disk. The information contained on the disks is part of this patent application and are incorporated into the specification by reference. One may use these tables in order to identify functional siRNAs for the gene provided therein, by simply looking for the gene of interest and an siRNA that is listed as functional. Preferably, one would select one or more of the siRNA that most optimized for the target of interest and is denoted as a pool pick.

Please amend the specification by replacing the paragraph on page 74, line 32, with the following amended paragraph:

Table XIII: ~~SMARTscores™~~ SMARTSCORES™ (siRNA RANKINGS)